Predicting Bioluminescence Signatures Based on the NAVOCEANO Bioluminescence Database

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LONG-TERM GOALS

Bioluminescence represents an operational threat to U.S. Navy nighttime operations because of the risk due to flow-stimulated light emission from naturally occurring plankton. Conversely, bioluminescence presents additional capabilities for detecting moving objects at night, particularly in the littoral zone where conventional acoustic surveillance is severely challenged. We are interested in the hydrodynamic conditions that stimulate bioluminescence, and the bioluminescence signature (footprint) that is created. To assess the risk due to flow-stimulated bioluminescence, the Naval Oceanographic Office (NAVOCEANO) measures bathyphotometer-stimulated bioluminescence potential throughout the worlds' oceans. The present study helps provide a scientific context for using the database to predict bioluminescence signatures of Navy interest. We are interested in how a luminescent wake scales with speed and bioluminescence potential, and how changes in bioluminescent potential change the spatial footprint and intensity of the bioluminescence signature.

OBJECTIVES

The primary goal of this project is to provide a biological characterization of the NAVOCEANO BIOLITE flow agitator as an initial step for establishing a transfer function between bioluminescence signatures and bioluminescence potential. The specific objectives of this study are:
1. For the BIOLITE flow agitator, measure bioluminescence potential as a function of dinoflagellate biomass for representative source organisms, and determine the range of agitator flow rates that are acceptable for operational use;
2. As a first step in predicting bioluminescence signatures based on bioluminescence potential, investigate bioluminescence signatures of a laboratory, turbulent jet as a function of cell concentration, species assemblage, and flow rate. In order to relate laboratory and field signatures we will determine a transfer function between BIOLITE-measured bioluminescence potential and that measured by a reduced flow agitator system more amenable for use in laboratory experiments. We use dinoflagellates as model organisms because they represent a major source of flow-stimulated bioluminescence in the littoral zone.
Questions addressed include:
1. What is an acceptable range of flow rates for agitator measurements of bioluminescence potential?
2. For each representative species, how does bioluminescence potential vary directly with abundance?
3. For each representative species and flow rate, how does the size and intensity of the luminescent jet (the signature) vary with bioluminescence potential (e.g., what effect does an order of magnitude increase in bioluminescence potential have on the bioluminescence signature)?

4. Can organism assemblages with equivalent bioluminescence potential generate different bioluminescence signatures?
This laboratory study provides a repeatable, quantitative, laboratory-based foundation for considering real-world scenarios.

APPROACH

Measurements of bioluminescence potential using the BIOLITE agitator were obtained as a function of organism species and abundance over a wide range of flow rates. For these tests a new computer-controlled flow system was assembled. Flow rates range from approximately 0.1 to 1 L/s, overlapping current operational flow rates that range from 0.75-1 L/s. Bioluminescence is detected by a photon-counting photomultiplier tube acquiring at 100 Hz. The contents are continuously stirred at 1 rpm prior to testing to gently mix the contents to promote a homogenous distribution of cells without stimulating bioluminescence. Each organism species is tested in replicate at each of three concentrations. A single experiment consists of a single concentration of a single species and a range of flow speeds. In separate tests the agitator is imaged with a low-light video camera to visualize the pattern of bioluminescence stimulation within the volume. Digitized frames are averaged to provide a qualitative description of the stimulation pattern.

Similar tests were done with the NOSC agitator, the prototype for the BIOLITE agitator. The NOSC agitator afforded a more two-dimensional flow field that is helpful in interpreting the BIOLITE flow visualization experiments. A low flow volume agitator, also based on the NOSC agitator design, was developed that can be cross-correlated with the NOSC and BIOLITE agitators and is more amenable for laboratory work.

An apparatus for generating standardized bioluminescence signatures was designed and fabricated. Bioluminescence generated by a turbulent jet is simultaneously measured by a photon-counting photomultiplier and imaged by a low-light video camera.

All experimental work is done by Latz, in consultation with collaborator Jim Rohr of SPAWAR Systems Center San Diego. Students from the University of California San Diego contributed towards apparatus fabrication, characterization, and testing, at no cost to the project.

WORK COMPLETED

For studying bioluminescence signatures, an apparatus was fabricated to produce jet turbulence. This project involved the assistance of engineering student Justin Vellido. The jet is formed by a 2 mm nozzle allowing filtered seawater to enter a vertical tank consisting of a known initial concentration of dinoflagellates. Flow rate is under computer control. Preliminary experiments showed that with increasing flow rate the volume of stimulated bioluminescence could extend beyond the field of view of the photomultiplier. Consequently it was necessary to enclose the entire jet reservoir within a 61-cm diameter integrating light chamber. The total flux of bioluminescence stimulated by the jet is measured by a photon-counting photomultipler and the jet is simultaneously imaged by a low-light video camera. Bioluminescence potential is measured by a low volume agitator system after each run of the jet.

RESULTS

According to current NAVOCEANO operational criteria (per communications with Mark Geiger of NAVOCEANO), BIOLITE measurements at flow rates < 0.75 L/s are discarded because they may not represent repeatable bioluminescence stimulation. Initial tests with the BIOLITE agitator with the dinoflagellates *L. polyedrum* and *P. fusiformis* indicated that within the range of flow rates of 0.5-1 L/s, levels of stimulated bioluminescence were similar (Fig. 1A). Also within this range average bioluminescence scaled with cell concentration. Maximum bioluminescence occurred at a flow rate of 0.2 L/s. Imaging of *P. fusiformis* bioluminescence within the agitator revealed bioluminescence distributed nearly evenly throughout the field of view of the camera (Fig. 1B) with erratic pathlines moving at speeds much less than that for the average incoming flow and often in the opposite direction (Fig. 1C). These images suggest that cells were stimulated not in the primary jet but in the recirculation zones occurring along the periphery of the jet.

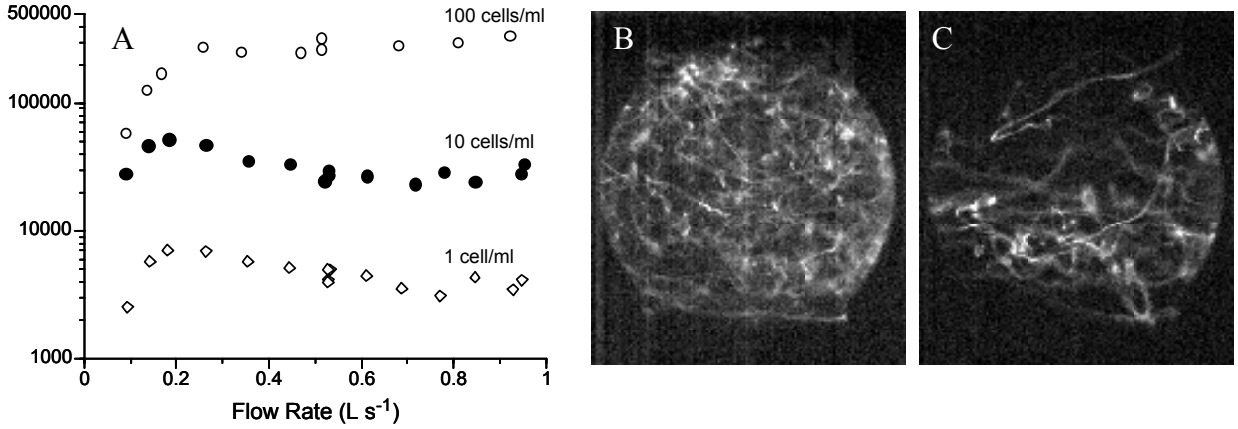

Figure 1. Bioluminescence stimulated by the BIOLITE agitator. (A) Average bioluminescence of three concentrations of L. polyedrum for a range of flow rates. Average bioluminescence scaled with cell concentration, and was maximal at 0.2 L/s. For each cell concentration average bioluminescence was similar for flow rates of 0.5-1 L/s. (B) P. fusiformis bioluminescence for a flow rate of 0.2 L/s and cell concentration of 0.1 cells/ml. The image is the average of 498 frames, each 25 ms in duration. Flow goes left to right. (C) Same, but averaging only 100 frames. These images suggest that bioluminescence is produced within the recirculation zones and not the primary jet.

The NOSC agitator, the prototype for the BIOLITE agitator, has a similar configuration, with entrance and exit ports 180˚ opposite each other, but its agitation chamber is disk shaped rather than cylindrical like the BIOLITE chamber. Thus its flow field can be considered a two-dimensional approximation of that for the BIOLITE agitator. Tests with *L. polyedrum* also showed average bioluminescence proportional to cell concentration. Average bioluminescence was similar for flow rates of 0.4-0.9 L/s (the highest tested). The flow pattern within the NOSC agitator is dominated with a central jet along centerline with recirculation zones above and below centerline (Fig. 2A). Imaging of *P. fusiformis* bioluminescence within the agitator reveals that most emission occurs within the recirculation zones (Fig. 2B).

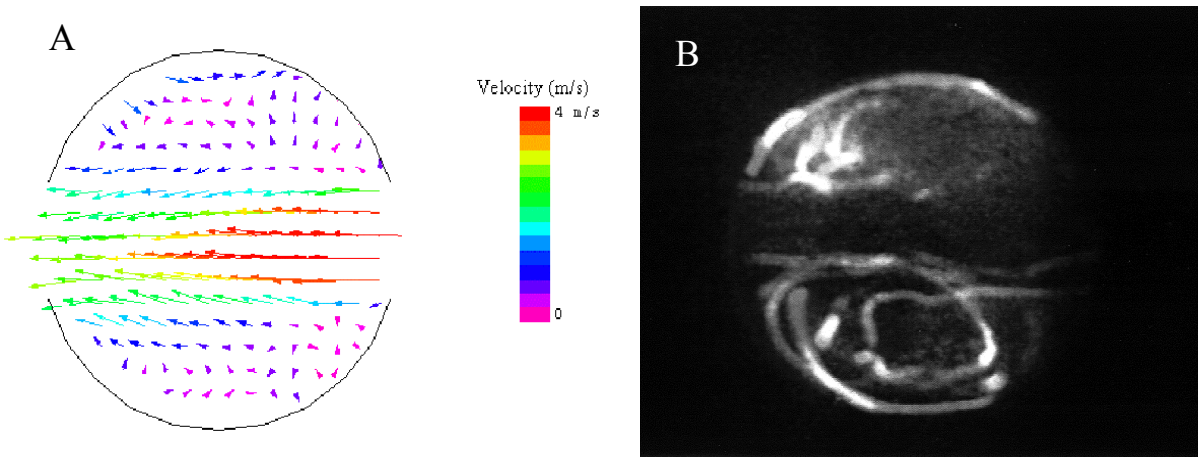

Figure 2. Flow pattern within the NOSC agitator. (A) Velocity field in the chamber midplane as measured by Laser Doppler Velocimetry for a flow rate of 0.4 L/s moving right to left. The flow consists of a primary jet with symmetrical recirculation zones above and below centerline. (B) Bioluminescence of P. fusiformis (concentration 1 cell/ml) at a flow rate of 0.13 L/s, with the flow moving left to right. The image represents the average of 10 frames, each 33 ms in duration. Most of the bioluminescence is associated with the recirculation zones and not the primary jet.

IMPACT/APPLICATIONS

Based on our initial results, it appears that BIOLITE measurements at flow rates of 0.5-1 L/s are similar for a given cell concentration. Therefore we recommend that the range of flow rates acceptable for NAVOCEANO requirements be extended down to 0.5 L/s.

We will continue to investigate the efficiency of agitator stimulation; because it appears that most measured bioluminescence is associated with recirculation zones and not the primary jet.

Of fundamental importance is how levels of flow-stimulated bioluminescence should be reported. Currently bioluminescence potential measured with the agitators is expressed per unit time, but this needs to be validated. If BIOLITE measurements were expressed per unit volume, then levels of bioluminescence potential would always be flow-rate dependent. The presence of recirculating flashes within the agitator volume complicates the understanding of the dependence of bioluminescence potential on flow rate.

Future tests with signatures from a turbulent jet will provide quantitative relationships between bioluminescence potential and bioluminescence signature size and intensity. Although it is apparent that measurements made by the BIOLITE and NOSC agitators scale with cell concentration, there is no established relationship between bioluminescence potential and bioluminescence signatures. By developing a laboratory scale flow agitator that can be correlated with NAVOCEANO's flow agitators, predictions/extrapolations of bioluminescent signatures for laboratory and oceanographic flows as well as flow fields of Navy interest (e.g., ship wakes) can be made, and in the case of laboratory flows, readily tested.

RELATED PROJECTS

This project involves the close collaboration with Jim Rohr, SSC San Diego, who provides expertise related to fluid mechanics and is an important link to NAVOCEANO. Mathematician Jeffery Allen, also at SSC San Diego, has assisted with the modeling. We are also collaborating with Edith Widder at Harbor Branch Oceanographic Institution in applying our experimental approaches to biological characterization of the HIDEX bathyphotometer.

REFERENCES

McDuffey, A. and J.L. Bird. 2002. The underway survey system at the Naval Oceanographic Office. Oceans 2002 Conference, Biloxi, MS, October 2002.

A web site focusing on laboratory activities and dinoflagellate bioluminescence, including a full listing of recent publications, is found at http://siobiolum.ucsd.edu. The site receives approximately 6000 hits per month from 50 countries.